The effect of acidic solutions and growth conditions on the adsorptive properties of bacterial surfaces

D. Borrok[a,*], J.B. Fein[a,1], M. Tischler[b,c,2], E. O'Loughlin[c,2], H. Meyer[c], M. Liss[c], K.M. Kemner[c,2]

[a] Department of Civil Engineering and Geological Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556, USA
[b] Department of Biological Sciences, Benedictine University, 5700 College Road, Lisle, IL 60532, USA
[c] Environmental Research Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA

Abstract

Data must be collected over a wide pH range to accurately model the adsorption of protons and metal onto bacterial surfaces; however, alterations in the structural and chemical properties of bacterial surfaces resulting from exposure to acidic solutions may affect the mechanisms of cation binding. Binding properties of bacteria may also be affected by nutrient and oxygen levels present during their growth. We measured Cd, Co, and Pb adsorption onto bacteria by using (1) bacteria washed with acidic solutions (pH ≈ 1.5), (2) non-acid-washed bacteria exposed to acidic parent solutions, and (3) non-acid-washed bacteria exposed to neutral parent solutions. The purpose was to determine the effect of acidic conditions on the adsorptive properties of *Pseudomonas mendocina*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Bacillus cereus*. We also measured Co adsorption onto bacteria (*Pseudomonas fluorescens*) grown under nutrient-rich and nutrient-limited conditions and onto the facultative bacterium *Shewanella oneidensis* MR-1 grown under aerobic and anaerobic conditions. Bacteria exposed to acidic solutions adsorbed more metals than bacteria not exposed to such solutions. We attribute the increase in adsorption to the irreversible displacement of structurally bound Mg and Ca by protons. After displacement, the protonated sites can participate in reversible metal adsorption reactions. Thermodynamic modeling suggests that concentrations of functional group sites on bacterial surfaces increase by as much as five times in response to acid washing, assuming that stability constants for the bacterial surface complexes remain the same. Although the sizes of the bacteria changed markedly in response to nutrient limits and oxygen content during growth, the mass-normalized extent of Co adsorption onto both *P. fluorescens* and *S. oneidensis* MR-1 was independent of growth conditions. We conclude that adsorption constants derived from experiments in which the bacteria are never exposed to acidic conditions probably provide the most accurate estimates of the extent of bacteria–metal adsorption in natural settings.
© 2004 Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: +1-574-631-4307; fax: +1-574-631-9236.

E-mail addresses: dborrok@nd.edu (D. Borrok), fein.1@nd.edu (J.B. Fein), Mtischler@ben.edu (M. Tischler), oloughlin@anl.gov (E. O'Loughlin), kemner@anl.gov (K.M. Kemner).

[1] Fax: +1-574-631-9236.
[2] Fax: +1-630-252-8895.

1. Introduction

The surfaces of bacteria contain organic acid functional groups that adsorb metal cations over a wide pH range (e.g., Beveridge and Murray, 1976, 1980). Because bacteria are abundant in virtually all near-

surface geologic systems, bacterial adsorption can contribute to the overall fate and transport of metals (Tornabene and Edwards, 1972; Warren and Ferris, 1998; Ledin et al., 1999; Tortell et al., 1999). Models that are capable of quantifying bacteria–metal adsorption are critical for predicting the mobility of metals under conditions not directly studied in the laboratory. Recently, surface complexation models (SCMs) have been invoked to describe the adsorption of metals onto individual functional group sites on the bacterial cell wall (Xue et al., 1988; Plette et al., 1995, 1996; Fein et al., 1997; He and Tebo, 1998; Fein, 2000; Haas et al., 2001). This modeling approach requires identification of the bacterial surface complexes, determination of the thermodynamic stability constants of those complexes, and determination of the concentrations of binding sites on the bacterial surface.

To constrain these parameters, adsorption measurements must be made over a wide pH range. However, some evidence suggests that exposure to relatively acidic conditions can alter the metal binding properties of bacteria. Chang et al. (1997) and Wong et al. (1993) observed an increase in adsorptive capacity in response to repeated acidification of the gram-negative bacterial species *Pseudomonas aeruginosa* and *Pseudomonas putida*, respectively. In their studies, adsorbed metals (Cu, Cd, or Pb) were driven from the bacterial surfaces by using aliquots of 0.1 M HCl to decrease the pH of the bacteria–metal suspensions to about 2.0. An increase in adsorptive capacity was observed when bacteria were resuspended in stock solutions rich in Cd (in the Chang et al. (1997) study) or Cu (in the Wong et al., 1993 study). The authors attributed the increase in metal adsorption to structural changes caused by the addition of HCl.

Other investigators have suggested that the concentrations of available nutrients during growth can affect the binding properties of bacterial surfaces (Cox et al., 1999). When nutrients are limited during growth, specific acidic functional group sites on bacterial surfaces may not develop. Nutrient levels can also induce changes in the size and shape of bacteria, but whether these changes affect the density of functional group sites on bacterial surfaces is unknown. Binding properties of facultative bacteria may be affected similarly by changing from an oxygen-rich to an anoxic environment during growth.

The effect of acid on bacterial surfaces is important, because most experiments examining the sorption of metals by bacteria involve exposure of bacteria to acidic conditions. For example, Yee and Fein (2001) observed that a number of gram-positive and gram-negative bacterial species exhibit similar extents of Cd adsorption. On the basis of this evidence they proposed that a universal set of thermodynamic stability constants could be used in an SCM to describe bacteria–metal adsorption in natural systems, where the determination of individual species abundances is difficult. However, in their study gram-positive bacteria were washed with acid (pH 1.5), and gram-negative bacteria were exposed to acidic parent solutions (pH < 2.8) prior to experimentation. Changes in the extent of bacteria–metal adsorption due to acid treatment may also have important implications for modeling bacterial adsorption in acidic natural environments and for industrial biosorption applications. In addition, bacteria–metal adsorption models can be applied in natural settings only if differences in growth conditions typical of those between laboratory and natural systems do not affect the binding properties of the bacteria.

Here, we report studies of bacteria–Cd, –Co, and –Pb adsorption. Starting solutions covered a range of pH values, and bacteria were acid-washed and non-acid-washed gram-negative (*Pseudomonas mendocina* and *P. aeruginosa*) and gram-positive (*Bacillus subtilis*, *Bacillus cereus*) species. We also conducted bacterial acidification experiments with several of the same species, and measured dissolved organic carbon (DOC) for a number of the acidification experiment samples to assess structural damage to bacterial cell walls. Additional bacteria–Co adsorption experiments were conducted with bacteria grown in nutrient-rich versus nutrient-limited solutions (*Pseudomonas fluorescens*) and aerobic versus anaerobic growth conditions (*Shewanella oneidensis* MR-1). The objectives of this study were (1) to determine if and how exposure to acidic solutions affects the concentrations of functional groups on bacteria surfaces and (2) to determine whether changes in nutrient and oxygen levels during growth of the selected bacteria affect their metal binding properties.

2. Materials and methods

2.1. Bacterial growth and harvest

Bacteria used in the experiments to test the effect of acid were initially cultured for 24 h at 32 °C in 3 ml of trypticase soy broth (TSB) with 0.5% yeast extract, then transferred to 1 l of broth of the same composition and cultured for another 24 h at 32 °C. The cells were removed from the nutrient medium by centrifugation and rinsed twice with 0.1 M $NaClO_4$ (the electrolyte used in the experiments). Acid-washed cells were prepared by suspension in 0.03 M HNO_3 (pH ≈ 1.5) for 30 min. The purpose of the acid wash step is to remove contaminant cations from the bacterial surfaces. Fig. 1A and B show that *B. subtilis* and *P. mendocina* cells, respectively, remain intact after the acid-washing step. Three additional rinses with 0.1 M $NaClO_4$ were then completed (for both acid-washed and non-acid-washed bacteria). After the final wash, cells were transferred to weighed test tubes and centrifuged for 1 h, with three pauses to decant the remaining electrolyte solution. The final moist/wet weight of each bacterial pellet was calculated and then converted to a dry weight, which is reported here. Previous work has shown that the wet/dry weight ratio is approximately 5:1 (Borrok et al., 2004), and that 1 g/l dry weight (which are reported here) corresponds to about 10^{10} cells/ml (Borrok and Fein, 2004a). The effect of nutrient concentration during growth was tested by using *P. fluorescens,* cultured and washed as described above except that Luria broth (LB) and diluted (10%) LB replaced TSB and the bacteria were grown at room temperature. *S. oneidensis* MR-1 was grown aerobically in TSB broth (as described above) and also in an anaerobic chamber (in the presence of nitrogen) to test the effect on cellular adsorptive properties of oxygen content during growth. Cells grown in the anaerobic chamber were harvested after reaching early stationary growth phase (about 7 days). Both aerobic and anaerobic bacteria were washed five times with 0.1 M $NaClO_4$.

2.2. Metal adsorption experiments

All metal adsorption experiments were conducted in batch reaction vessels. Cadmium adsorption experiments were conducted with *B. subtilis, B. cereus, P. aeruginosa,* or *P. mendocina* at bacterial concentrations of 1.0 g/l. Additional Cd experiments were conducted with *B. subtilis* at 0.05 g/l and *P. mendocina* at 0.3 g/l. Cobalt adsorption experiments were conducted with *B. subtilis*, *P. fluorescens* (LB and 10% LB), and *S. oneidensis* MR-1 (aerobic and anaerobic), all at 1.0 g/l. The Pb adsorption experiment used *B. subtilis* at 0.05 g/l. All experiments were conducted with the chosen metal at 10 ppm (diluted from a 1000-ppm atomic absorption standard), except that the experiment with Cd and *B. subtilis* at 0.05 g/l used Cd at 5.4 ppm. The chosen concentrations of metal
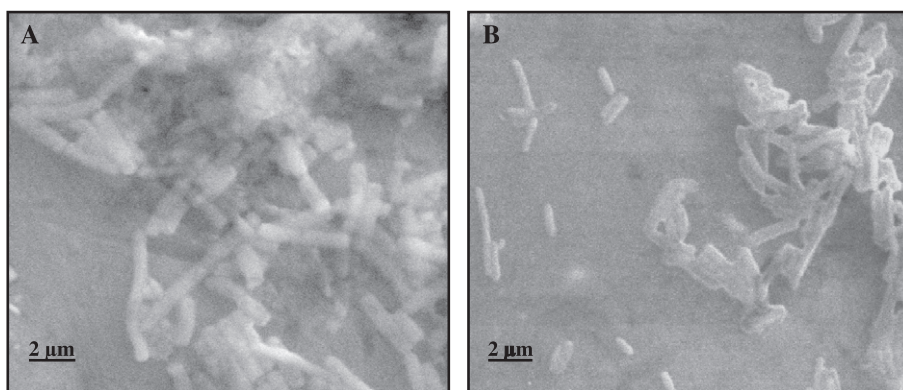

Fig. 1. Scanning electron microscope (SEM) images of acid-washed *B. subtilis* (A) and *P. mendocina* (B) cells. Photographs show clumps of intact cells that are similar in size and shape to cells that have not been acid-washed. The cells were prepared for imaging by suspension in 0.1 m $NaClO_4$ electrolyte. Minute aliquots of the suspension were allowed to dry on metallic stubs with no additional treatment. Images A and B were taken under ultra-high vacuum at 20 kv at 12,400 × and 10,280 × magnification, respectively.

and bacteria are high to improve accuracy in our experiments; however, the bacteria/metal ratios were similar to those found in some natural and industrial settings. Two types of stock solutions were made: (1) those in which the bacteria were added prior to pH adjustment (termed here "acidic parent solutions" or APS) and (2) those adjusted to pH 6–8 prior to the addition of the bacteria (termed here "neutral parent solution" or NPS). The pH of the APS stock solutions ranged from 2.6 to 2.8 after the addition of bacteria.

Three types of experiments were conducted using *B. subtilis* and *P. mendocina*: (1) bacteria were washed with acid and suspended in an acidic parent solution ("Acid Wash"), (2) bacteria were not washed with acid but were suspended in an acidic parent solution ("APS"), and (3) bacteria were not washed with acid and were suspended in a neutral parent solution ("NPS"). Acid Wash and NPS (but not APS) experiments were performed with *P. aeruginosa* and *B. cereus*. The Co adsorption experiments using *P. fluorescens* and *S. oneidensis* MR-1 were conducted according to the APS procedure.

Known masses of bacteria were suspended in the chosen parent solution and stirred gently until the distribution was homogeneous. Aliquots (approximately 10 ml) of the bacteria–metal–electrolyte suspensions were transferred into individual batch reaction vessels. The pH of each batch experiment was adjusted to the desired value by using a small volume of concentrated NaOH or HNO$_3$. Each batch experiment was then allowed to equilibrate on a rotating rack for 2 h, and the final (equilibrium) pH was measured. Previous kinetics experiments showed that most bacterial adsorption reactions equilibrate within 30 min (Fein et al., 1997; Yee and Fein, 2001). Each sample was then centrifuged, filtered through a 0.45-μm nylon syringe filter membrane, acidified to prevent precipitation, and analyzed for the dissolved metal by using an inductively coupled plasma-atomic emission spectroscopy (ICP-AES) technique. Calibration standards were made with the same electrolyte used in the experiments. Analytical uncertainty was less than approximately ± 2%. The decrease in aqueous metal concentration during the experiment was due to metal adsorption onto the bacterial cell wall. Control experiments in our laboratory have demonstrated that metal adsorption onto

the experimental apparatus is negligible (data not shown).

2.3. Acidification and dissolved organic carbon experiments

Acidification experiments measured the concentration ranges of exchangeable metals associated with the bacterial surfaces after completion of the incubation and washing procedure. In these experiments, we suspended 1.0 g/l of non-acid-washed bacteria in a 250-ml Teflon reactor vessel with 0.1 M NaClO$_4$ electrolyte (and no added metals). The pH of the electrolyte–bacteria mixture was adjusted downward from its initial nearly neutral value by using small amounts of concentrated HNO$_3$. The suspension was stirred vigorously with a floating stir bar to ensure homogeneity. After each downward desorption step, 10-ml aliquots of suspension were transferred to a separate reaction vessel. After equilibration for 2 h on a rotating rack, the final pH was measured, and the samples were filtered and analyzed for a range of dissolved metals. Initial analysis of the solutions was conducted by using an inductively coupled plasma-mass spectrometer (ICP-MS). Analytical uncertainty was less than approximately ± 4%. On the basis of these results, additional analyses for Mg and Ca were performed using the ICP-AES technique described above.

On several of the samples collected during the acidification experiments, DOC analyses were also performed as a rough estimate of the extent of structural damage to the bacterial cell walls. Analyses were performed with a Shimadzu 5000 DOC analyzer, and calibration standards were made by diluting a potassium hydrogen phthalate standard containing carbon at 1000 ppm with the electrolyte used in the experiments. Experimental error was less than ± 3%.

3. Results

The results of the Acid Wash, APS, and NPS experiments with *B. subtilis* and *P. mendocina* are presented in Figs. 2 and 3, respectively. All three types of experiments for each bacterial species exhibit different adsorption edges. For each bacterial species, the adsorption edges corresponding to the Acid Wash
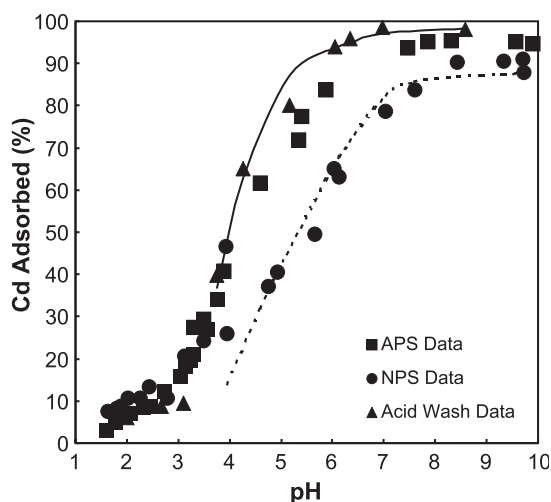
Fig. 2. Cd adsorption data for Acid Wash, NPS, and APS experiments with *B. subtilis* at 1 g/l. Corresponding surface complexation model fits for Acid Wash and NPS data are shown with solid and dashed lines, respectively.

experiments occur at the lowest pH values, while the adsorption edges associated with the NPS experiments occur at the highest pH values. For example, in the *B. subtilis* experiments, 50% Cd ads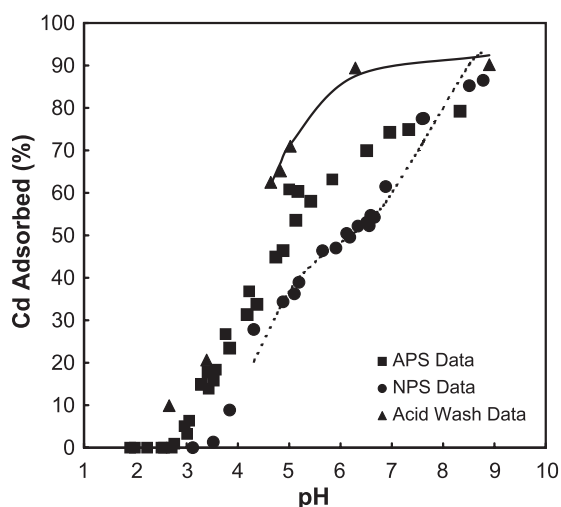orption occurs at pH 4.0 for acid-washed bacteria, but for the APS and NPS experiments, 50% Cd adsorption occurs at pH 4.2 and pH 5.4, respectively (Fig. 2). The pH values at 50% Cd adsorption for the *P. mendocina* experiments are 4.2, 5.0, and 6.2, for the Acid Wash, APS, and NPS, experiments, respectively (Fig. 3). However, with increasing pH, the extent of adsorption measured in the *P. mendocina* APS experiment approached the NPS results, and at the highest pH conditions studied, the NPS experiment yielded more adsorption than the APS experiment. This pattern was not observed in the *B. subtilis* experiments. The extent of Co adsorption onto *B. subtilis* in the Acid Wash and NPS experiments is presented in Fig. 4. As observed in the Cd experiments, the Co adsorption edge generated with acid-washed bacteria occurred at a lower pH than the NPS adsorption edge. However, there was little difference between the Acid Wash and NPS results for Pb and Cd sorption onto *B. subtilis* at 0.05 g/l (Figs. 5 and 6, respectively).

The results of the acidification and DOC experiments with *B. subtilis* and *P. mendocina* are presented in Figs. 7 and 8, respectively. As pH conditions became acidic, Mg and Ca were liberated from both bacterial species. The DOC concentration in solution increased only slightly over the entire pH range for *B. subtilis*, but it increased dramatically with decreasing pH for *P. mendocina*.

Fig. 3. Cd adsorption data for Acid Wash, NPS, and APS experiments with *P. mendocina* at 0.3 g/l. Corresponding surface complexation model fits for Acid Wash and NPS data are shown with solid and dashed lines, respectively.
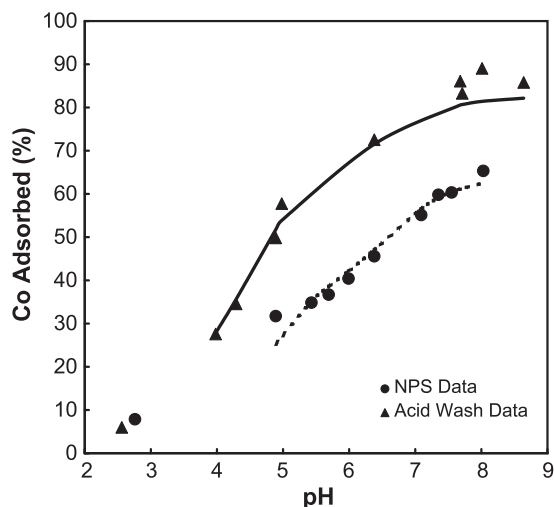

Fig. 4. Co adsorption data for Acid Wash and NPS experiments with *B. subtilis* at 1 g/l. Corresponding surface complexation model fits for Acid Wash and NPS data are shown with solid and dashed lines, respectively.
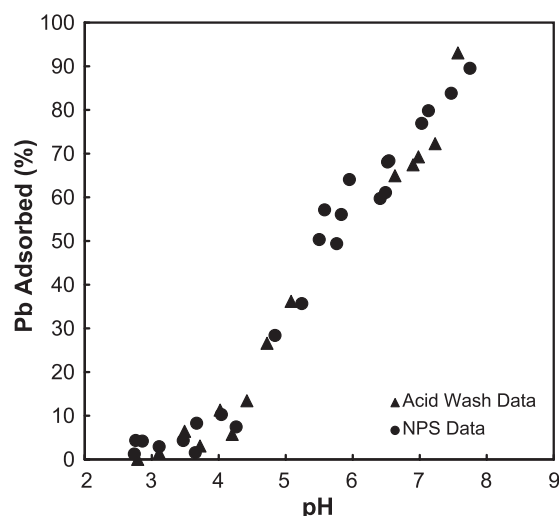
Fig. 5. Pb adsorption data for Acid Wash and NPS experiments with *B. subtilis* at 0.05 g/l and Pb at 10 ppm (48 μmol/l).

The Co adsorption data for *P. fluorescens* grown in diluted LB medium match (within experimental uncertainty) the Co adsorption data for *P. fluorescens* grown in regular LB medium (Fig. 9), suggesting that nutrient levels do not have a significant effect on Co adsorption by this microbe. In addition, the Co adsorption data for anaerobically and aerobically grown *S. oneidensis* MR-1 demonstrate that oxygen content
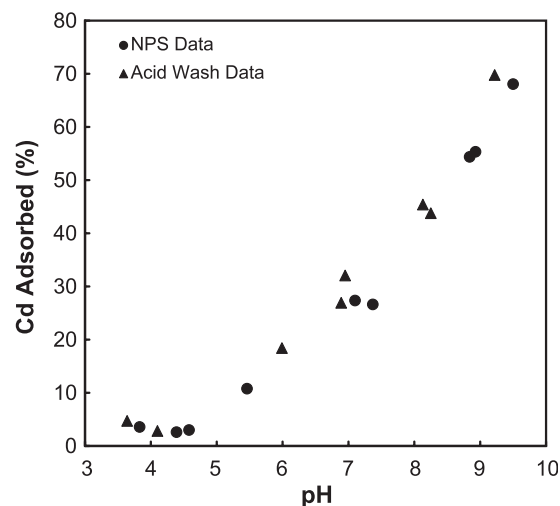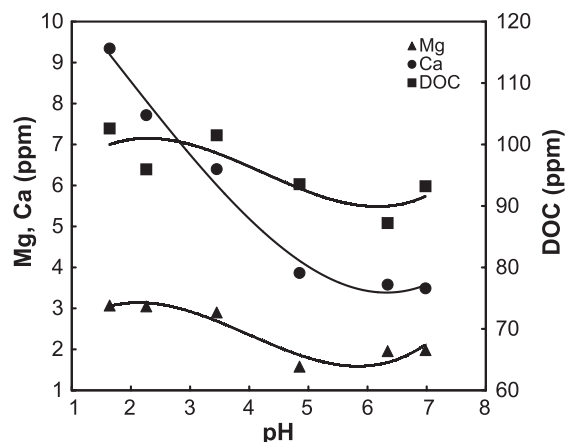

Fig. 7. Concentration data and best-fit curves for Mg, Ca, and DOC as a function of pH, with *B. subtilis* at 1 g/l.

during growth does not significantly affect the extent of Co adsorption onto *S. oneidensis* MR-1 under the experimental conditions (Fig. 10).

Results from the additional Acid Wash and NPS experiments with Cd and *B. subtilis*, *B. cereus*, *P. aeruginosa*, and *P. mendocina* are presented in Fig. 11. In all cases the adsorption edges generated in the Acid Wash experiments occur at lower pH than do the adsorption edges for the NPS experiments. However, the difference is greatest for the gram-positive bacte-

Fig. 6. Cd adsorption data for Acid Wash and NPS experiments with *B. subtilis* at 0.05 g/l and Cd at 5.4 ppm (48 μmol/l).
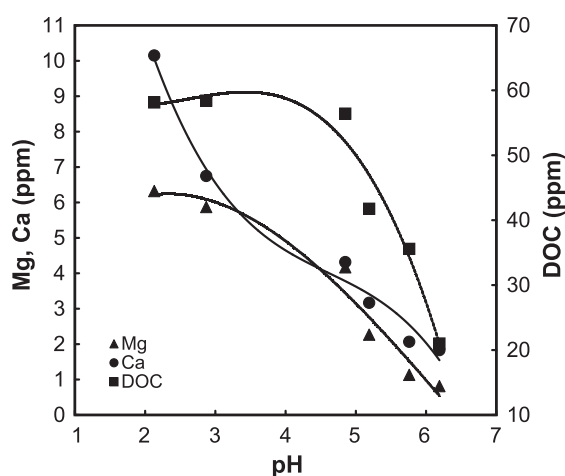

Fig. 8. Concentration data and best-fit curves for Mg, Ca, and DOC as a function of pH, with *P. mendocina* at 1 g/l.

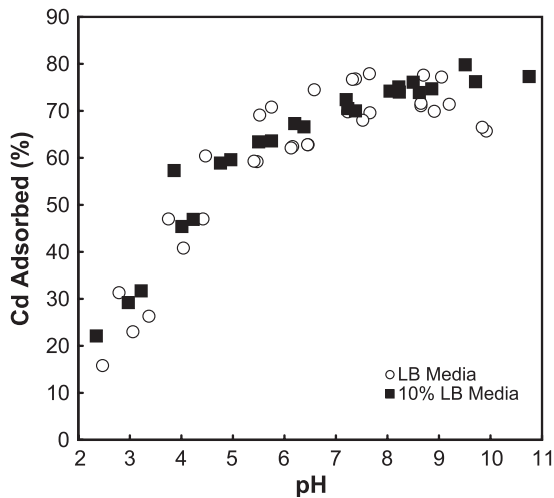Fig. 9. Co adsorption data for experiments with *P. fluorescens* (1 g/l) grown in full LB medium and 10% LB medium.

Fig. 11. Cd adsorption data for Acid Wash and NPS experiments with *B. subtilis*, *B. cereus*, *P. mendocina*, or *P. aeruginosa* at 1 g/l. Data are compared to the ''universal adsorption edge'' (solid line) defined by Yee and Fein (2001).

rium *B. cereus*. In the *B. cereus* experiments, 50% Cd adsorption occurs at pH 3.7 in the Acid Wash experiment, but at pH 5.7 in the NPS experiment. At the highest pH studied, the results from the Acid Wash experiments with the gram-negative species *P. aeruginosa* and *P. mendocina* fail to reach the maximum
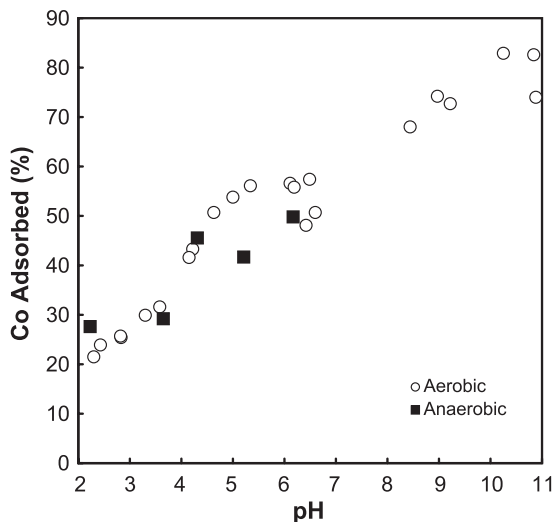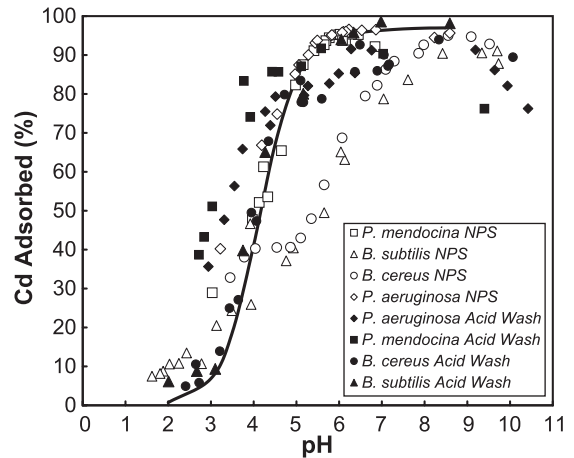

Fig. 10. Co adsorption data for experiments with *S. oneidensis* MR-1 (1 g/l) grown under aerobic and anaerobic conditions.

extents of adsorption observed in the NPS experiments with these same species.

4. Discussion

4.1. Effect of acid on the adsorptive properties of bacterial surfaces

B. subtilis and *P. mendocina* were subjected to acidic conditions in both the Acid Wash and APS experiments. The purpose of the Acid Wash treatment is to remove any adsorbed metals from the cell wall prior to introduction of the experimental metal of interest. Bacteria in the APS experiments were not washed with acid, but were subjected to acidic (pH ≈ 2.8) stock solutions. Hence, the difference between the Acid Wash and the APS experiments was that contaminant cations removed through acid washing would be retained in the APS experimental systems. Differences between the APS and the Acid Wash experimental results suggest that the electrolyte rinses were insufficient to remove all of the metals adsorbed from the growth media. Competition for binding sites between the growth medium metals and the experimental metal (Cd) probably resulted in slightly less adsorption of Cd

in the APS experiments than in the Acid Wash experiments (Figs. 2 and 3).

The acidification experiments demonstrate which metals (other than the experimental metal) were present in solution during the APS experiments. An initial ICP-MS multi-element scan indicated that only Mg and Ca were present at concentrations greater than 1 ppm. Subsequent acidification experiments suggested that maximum levels of approximately 3 ppm for Mg and 9 ppm for Ca were present in solution during the *B. subtilis* APS experiment (Fig. 7), while maximum levels of approximately 6 ppm for Mg and 10 ppm for Ca were present in solution during the *P. mendocina* APS experiment (Fig. 8).

During the acidification experiments, DOC was monitored to determine whether the release of Mg and Ca was due to desorption of metal (not structurally bound) from the bacterial surfaces or to the possible structural breakdown of cells. Calcium and Mg are structural components within the cell walls of gram-positive bacteria and are the major constituent metals in the outer membranes of gram-negative species (Beveridge and Koval, 1981; Beveridge, 1989). The DOC level remained relatively constant over the entire pH range of the experiment with *B. subtilis* (Fig. 6); however, DOC increased with decreasing pH in the experiment with *P. mendocina* (Fig. 8), suggesting that the acidic conditions caused some damage to the *P. mendocina* cells. Fig. 1A and B show that *B. subtilis* and *P. mendocina* cells remain intact after exposure to acidic solutions, suggesting that cell damage did not result in cell lyses. However, damage to *P. mendocina* cells may have included the loss of proteins or lipopolysaccharides in the cells outer membrane brought about by the displacement of structurally bound Mg and Ca (Beveridge and Koval, 1981). Under acidic conditions, the driving force of protonation appears to be great enough to displace structurally bound Mg and Ca from some bacteria.

Unlike the adsorption edge generated by the *B. subtilis* APS experiment, the APS adsorption edge generated with *P. mendocina* crosses the NPS data at high pH. We attribute part of this Cd adsorption behavior to the added DOC in solution. DOC values increased by more than 200% in the acidification experiment with *P. mendocina* (Fig. 8), and this DOC might compete with the bacterial cell wall in binding Cd. Hence, aqueous complexation of Cd with

DOC decreases the extent of Cd adsorption onto the non-dissolved bacterial fraction. The effect of this competition is most noticeable at higher pH, where the DOC would be more negatively charged. This effect was observed only in experiments with gram-negative bacteria, suggesting that destruction of the outer membrane material is responsible for the release of DOC. This conclusion is consistent with the results of Beveridge (1989), who described the release of blebs of outer membrane material from gram-negative bacteria in response to displacement of structurally bound Mg and Ca.

With both *B. subtilis* and *P. mendocina*, the adsorption edges occurred at higher pH values (by 1–2 units) in the NPS experiments than in the APS experiments (Figs. 2 and 3). The total concentrations of Mg + Ca available for competition with Cd were identical in NPS and APS experiments (because the Mg and Ca were not released and rinsed away prior to either experiment). Therefore, competition of Mg + Ca for binding sites cannot account for this shift. A similar shift occurs for the NPS adsorption edge relative to the Acid Wash adsorption edge for Co adsorption onto *B. subtilis* (Fig. 4). Subjecting bacteria to acidic solutions appears to significantly increase the number of cell wall functional group sites that are available for metal binding. We further hypothesize that during the NPS experiments, Mg and Ca were structurally and irreversibly bound to specific functional groups, rendering these sites unable to adsorb Cd and shifting the adsorption edge to higher pH values. However, once the NPS solution was subjected to acidic conditions (for the experiments conducted at pH values less than approximately 4), Mg and Ca broke free from these functional groups, allowing the sites to compete freely to adsorb Cd. This explanation accounts for the confluence of the NPS, APS, and Acid Wash experimental data below approximately pH 4. These observations contradict the assumption that all metal adsorption reactions involving bacteria are fully reversible. However, adsorption reactions do appear to be fully reversible once structurally bound metals have broken free during acid washing or interaction with acidic solutions (Fowle and Fein, 2000). In other words, it is the initial breaking free of Mg + Ca that is an irreversible process. It is also possible that the loss of divalent cations from within the bacterial surface has an appreciable effect on the overall electronegativity of the

bacteria. A larger negative charge could result in increased affinity for metal binding, which would also be consistent with our results.

In contrast to the results presented above, the Cd and Pb adsorption edges from the Acid Wash and NPS experiments with only *B. subtilis* at 0.05 g/l are not significantly different (Figs. 5 and 6). We hypothesize that the metals in these experiments exerted the same effect on the bacterial cell wall as did protons in the previous experiments. In both experiments, an extremely high molar ratio of metal to bacteria was used (about 1 mmol/g). The surface sites available for binding metals became saturated at these high metal/bacteria ratios. This saturation apparently created conditions for enhanced adsorption, even in the absence of acidification, most likely by liberating Mg and Ca from the bacterial surface and thereby creating additional sites for metal uptake. However, spectroscopic evidence is clearly necessary to verify this proposed mechanism.

## 4.2. Effect of growth conditions on binding properties of bacterial surfaces

The same bacterial species appear to adsorb similar extents of metals, regardless of the richness of their growth media (Fig. 9) or (in the case of facultative bacteria) the availability of oxygen (Fig. 10). Scanning electron microscope (SEM) images of *P. fluorescens* bacteria grown under nutrient-limited conditions (10% LB medium) showed that they retained their rod-shaped character, but were only a fraction of the length of *P. fluorescens* cells grown in full-strength LB. Despite this size difference, there was no significant difference in the extent of Co adsorption onto each bacterial population (Fig. 9). Similar size differences were observed for *S. oneidensis* MR-1 grown in aerobic versus anaerobic conditions. However, despite these changes, both anaerobic and aerobic *S. ondeidensis* MR-1 adsorbed Co to similar extents (Fig. 10). These preliminary results suggest that changes in growth conditions have little effect on the metal adsorption properties of bacterial surfaces.

4.3. Modeling of bacteria–metal adsorption reactions

For comparison with previously published results and to serve as a basis for comparison to the NPS adsorption edges in this study, the Acid Wash experimental results were modeled by using an SCM approach. The NPS experimental results were modeled to develop a set of thermodynamic constants that reflect realistic (circumneutral pH) geologic conditions, as well as to quantify the changes in functional group site concentrations and adsorption equilibrium constants that result from acid exposure. The APS experimental results were not modeled, because the structurally bound cations released into solution during these experiments compete with the experimental metal for adsorption to functional group sites. The results from the two experiments conducted at high metal/bacteria ratios also were not modeled, because the Acid Wash and NPS results were nearly identical.

The SCM approach uses mass action equations that describe the dominant chemical reactions taking place on the bacterial cell wall, in conjunction with mass balance constraints on the system, to define the concentrations of individual surface and aqueous species. We represent the deprotonation of a specific type of organic functional group on the bacterial surface with the following reaction:

$$\mathrm{R-A}H^{\circ} \Leftrightarrow \mathrm{R-A}^{-} + H^{+}, \tag{1}$$
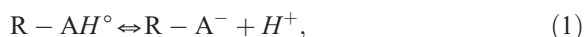
where R is the bacterium to which functional group type A is attached. The number of discrete surface binding sites A, the concentration of each of these sites, and their proton adsorption constants have previously been determined through modeling of potentiometric titration data. Fein et al. (2004) developed a four-site non-electrostatic model for proton binding onto *B. subtilis* with protonation constant (pK_a) values of 3.3, 4.8, 6.8, and 9.1. Borrok and Fein (2004b) developed a similar four-site non-electrostatic model for proton binding onto *P. mendocina* with pK_a values of 3.4, 4.7, 6.5, and 9.3.

To develop our model, we describe metal adsorption onto the bacterial surface as an interaction between deprotonated surface sites and aqueous metal cations, M^{m+}, as follows:

$$\mathrm{M}^{m+} + \mathrm{R-A}^{(-1)} \Leftrightarrow \mathrm{R-A(M)}^{(m-1)}. \tag{2}$$
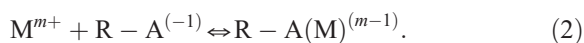
The corresponding mass action equation is

$$K = \frac{[\mathrm{R-A(M)}^{(m-1)}]}{[a_{\mathrm{M}^{m+}}][\mathrm{R-}A^{-}]} \tag{3}$$

where K is the equilibrium constant for Reaction (2), the brackets represent the concentrations of functional group sites in mol/l, and $a_{M^{m+}}$ is the activity of the aqueous metal cation.

Values for the acidity constants (equilibrium constants for Reaction (1)) and site concentrations for the bacterial surfaces used in these experiments are known (Borrok and Fein, 2004b; Fein et al., 2004); therefore, if we measure the absolute concentrations of adsorbed and total metal at a particular pH, we can use the experimental data to constrain the value of the equilibrium constant for Reaction (2). Calculations were performed with the program FITEQL 2.0 (Westall, 1982), using a non-electrostatic model. A number of previous studies have employed constant capacitance electrostatic models to quantify the electrostatic surface effects (e.g., Fein et al., 1997; Daughney et al., 1998, Yee and Fein, 2001). However, the high capacitance value (8.0 F/m^2) used in these studies is nearly equivalent to using a non-electrostatic model. Hence, the modeling parameters developed in this study are comparable to previous studies. The best-fit model for the experimental data was determined by testing model fits for different combinations of bacterial surface complexes $(R-A(M)^{(m-1)}$ in Eq. (2)). The relative goodness of fit of each tested model was quantified by using the residual function $V(Y)$ from the FITEQL 2.0 output (Westall, 1982). Because Acid Wash and NPS data points converge under acidic conditions (below about pH 4.0), we neglect data points in this range in our models.

Adsorption of metals onto two separate functional group sites provided a best fit to the *B. subtilis* and *P. mendocina* Acid Wash data. Models that excluded either one of these sites did not fit the data over the entire pH range. Because low pH data points were neglected, metal binding onto the lowest pK_a site for each bacterium was not necessary. The binding stoichiometries for these models are compiled in Table 1, while the model fits are presented with the data in Figs. 2, 3, and 4. The calculated metal adsorption constants for Cd (log $K = 4.2$) and Co (log $K = 3.7$) adsorption onto site 2 (p$K_a = 4.8$) of *B. subtilis* compare favorably with those reported previously for similar experimental conditions (for Cd, log $K = 4.0$ [Yee and Fein, 2001]; for Co, log $K = 3.5$ [Fein et al., 2001]). The adsorption constant for site 2 (p$K_a = 4.7$) for Cd adsorption onto *P. mendocina*

Table 1
Adsorption constant values for best-fit surface complexation models

Bacterium and experiment	Metal adsorption constant (log K)				
	Site 1 (pK_a 3.2)	Site 2 (pK_a 4.8)	Site 3 (pK_a 6.8)	Site 4 (pK_a 9.1)	$V(Y)$ value
B. subtilis – Cd					
Acid Wash	NA	4.22	5.51	NA	3.23
NPS	NA	3.43	4.59	NA	15.3
B. subtilis – Co					
Acid Wash	NA	3.75	4.20	NA	30.5
NPS	NA	3.14	3.91	NA	2.54
	Site 1 (pK_a 3.4)	Site 2 (pK_a 4.7)	Site 3 (pK_a 6.5)	Site 4 (pK_a 9.4)	$V(Y)$ value
P. mendocina – Cd					
Acid Wash	NA	4.42	5.41	NA	0.98
NPS	NA	3.76	4.46	NA	22.3

All adsorption constants are for monodentate metal adsorption reactions onto deprotonated functional group sites (Reaction (2)). NA = Not applicable for best-fit model.

(log $K = 4.4$) is comparable to that for Cd binding onto *B. subtilis*.

We used two approaches for modeling the NPS data. In one approach, we assumed that the values of the Cd adsorption constants were identical to those derived from the Acid Wash models, and we allowed the concentrations of functional group sites to vary to achieve the best fit to the data. This approach was used to determine the maximum number of functional group sites that become available for metal binding after structurally bound Mg and Ca are displaced by protons. Alternatively, the NPS data were also modeled by using the same reaction stoichiometries as in the Acid Wash model and the same site concentrations, but then solving for new Cd adsorption constants. This approach determined the hypothetical change in metal binding affinity caused by acid washing, under the assumption that no new functional group sites were created. This type of modeling cannot probe the exact mechanisms involved that lead to increased binding capacity in response to acid. Hence we cannot rule out either of the proposed mechanisms (increased sites vs. increased binding affinity).

Model fits for the cases where Cd and Co adsorption constants, as determined in the Acid Wash experi-

ments, were held constant and site concentrations were allowed to vary are presented in Figs. 2, 3, and 4. The concentrations of functional group sites increased in response to acid washing by four and five times for Cd adsorption onto *P. mendocina* and *B. subtilis*, respectively. For Co adsorption onto *B. subtilis*, the increase in functional group site concentration was about three times.

The concentrations of additional acid-activated sites (normalized to the mass of bacteria) created among these three examples averaged about 1.1×10^{-3} mol/g. This number is about three times the maximum concentrations of Mg and Ca released during acid washing (Figs. 7 and 8). This finding may suggest that the structural metals are bound in multi-dentate fashion, with a number of functional groups attached to each divalent cation. Alternately, displacement of Mg and Ca by protons may not only increase the concentration of binding sites but also alter their overall affinity for binding. Clearly, spectroscopic measurements are required to distinguish between these possible end members. X-ray adsorption fine structure (XAFS) analysis of acid-washed and non-acid washed bacterial surface–metal complexes could be used to determine the nature of the acid-activated binding sites. If the acid-activated binding sites are chemically different than the original functional group binding sites, it would suggest they likely have different affinities for binding metals.

When functional group site concentrations were held constant in the modeling, we obtained equally good fits to the data as when the functional group site concentrations were allowed to vary. As shown in Table 1, the calculated best-fitting Cd and Co adsorption constants in this modeling approach are significantly lower than those obtained though modeling of the corresponding Acid Wash data.

4.4. Implications for describing bacteria–metal adsorption in nature

Potentiometric titration data demonstrate that most, if not all, bacterial species exhibit significant buffering capacity, even at very low pH (Fein et al., 1997; Martinez et al., 2002; Plette et al., 1995). Therefore, proton and metal adsorption data are needed at acidic pH to constrain the binding behavior of the functional groups that are proton-active at low pH. However, our study suggests that exposure to acidic pH conditions irreversibly alters the bacterial cell wall, increasing proton and metal binding capacity by displacing structurally bound Mg and Ca. Hence, experiments with bacteria in acidic conditions probably overestimate the extent of adsorption in natural systems, where bacteria grow at circumneutral pH. Experiments like those conducted by Yee and Fein (2001) to define the "universal adsorption edge" need to be revised if the results are to reflect the extent of metal adsorption under near-neutral conditions. Fig. 11 depicts the results of NPS and Acid Wash experiments on a number of gram-positive and gram-negative bacteria, in conjunction with the "universal adsorption edge" determined under Acid Wash and APS experimental conditions by Yee and Fein (2001). Contrary to the universal adsorption behavior documented by Yee and Fein (2001) for "acid-activated" bacteria, we found significant differences in the extent of adsorption for gram-negative and gram-positive bacteria under natural pH (NPS) conditions. However, the gram-positive bacteria appear to have similar maximum, or acid-activated, functional group site concentrations. As in our study, Mullen et al. (1989) showed that the gram-negative bacteria *Escherichia coli* and *P. aeruginosa* adsorbed more Cd than the gram-positive species *B. subtilis* and *B. cereus*. Our NPS results show that the gram-negative species tested here adsorb about 30% more Cd than do the gram-positive species at pH 6.0. The differences in adsorptive capacity are probably due to fundamental differences in the structures of the bacterial cell walls (Beveridge and Koval, 1981). This gap in adsorption was obscured in the Yee and Fein (2001) study, because acid washing of the gram-positive bacteria increased their adsorptive capacity to a level resembling that of the gram-negative bacteria. At high pH, the acid-washed gram-negative bacteria in Fig. 11 exhibited a loss of adsorptive capacity, in comparison to the NPS results. Apparently, the functional groups responsible for binding at high pH (perhaps the outer membrane material) were partly washed away during acid treatment, resulting in lower overall adsorption.

The development of a true "universal" model that can describe bacteria–metal adsorption over the range of pH conditions in the environment will require a more mechanistic understanding of cell wall structural

changes that occur in response to exposure to acidic conditions. For now, bacteria–metal adsorption in circumneutral aqueous settings seems to be estimated best by subtracting approximately 1 log unit from previously published Cd adsorption constant values (Yee and Fein, 2001) and approximately 0.5 log units from previously published Co adsorption constant values (Fein et al., 2001). Metals with adsorption constants similar to those of Cd and Co would probably require similar corrections; however, whether adsorption constants for other metals with higher affinities for adsorption (e.g., Pb, Cu, Al) are affected similarly is not known. At extremely high metal/bacteria ratios the effect of acid appears to diminish (Figs. 5 and 6), negating the need for correction.

5. Summary and conclusions

When bacteria are exposed to acidic solutions, structurally bound Mg and Ca are displaced by protons, resulting in increased metal binding capacity for the bacteria (Figs. 2, 3, and 4). Modeling results demonstrate that acid washing of bacteria, inducing the release of Mg and Ca, may cause a 3- to 5-fold increase in the concentration of effective functional group sites. The cell wall of the gram-negative species *P. mendocina* appears to be disrupted by the displacement of Mg and Ca during acidification, resulting in an increase in the release of DOC. Displacement of Mg and Ca in the outer membrane has previously been shown to induce sloughing off of organic material (Beveridge, 1989). This organic material can compete with bacterial cell walls for the adsorption of metals. Minor changes in the size of *P. fluorescens* and *S. oneidensis* MR-1 were observed in response to changes in the concentrations of nutrients and oxygen, respectively, in the growth medium; however, metal binding properties of the bacteria appeared to be unaffected by these changes.

Our study demonstrates that adjustments to bacteria–metal adsorption data generated under acidic conditions may be necessary if the results are to be applied to natural settings. Our results also suggest that treating bacteria with acid before they are used in remediation scenarios might significantly improve their performance as adsorbents for some metals. Preliminary work also indicates that the concentration

of nutrients during growth of bacteria and the presence or absence of oxygen during growth of facultative bacteria have little effect on their metal binding properties.

References

Beveridge, T.J., 1989. Role of cellular design in bacterial metal accumulation and mineralization. Annu. Rev. Microbiol. 43, 147–171.

Beveridge, T.J., Koval, S.F., 1981. Binding of metals to cell envelopes of *Escherichia coli* K-12. Appl. Environ. Microbiol. 42, 325–335.

Beveridge, T.J., Murray, R.G.E., 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. J. Bacteriol. 127, 1502–1518.

Beveridge, T.J., Murray, R.G.E., 1980. Sites of metal deposition in the cell wall of *Bacillus subtilis*. J. Bacteriol. 141, 876–887.

Borrok, D., Fein, J.B., 2004a. The distribution of protons and Cd between bacterial surfaces and dissolved humic substances determined through chemical equilibrium modeling. Geochim. Cosmochim. Acta 68, 3043–3052.

Borrok, D., Fein, J.B., 2004b. Ionic Strength Dependence of Adsorption Reactions for Protons, Pb, and Cd onto Two Gram Negative Aerobic Bacteria. J. Colloid Interface Sci. (submitted for publication).

Borrok, D., Fein, J.B., Kulpa Jr., C.F., 2004. Proton and Cd adsorption onto natural bacterial consortia: testing universal adsorption behavior. Geochim. Cosmochim. Acta (in press).

Chang, J., Law, R., Chang, C., 1997. Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. Water Res. 31, 1651–1658.

Cox, J.S., Smith, D.S., Warren, L.A., Ferris, F.G., 1999. Characterizing heterogeneous bacterial surface functional groups using discrete affinity spectra for proton binding. Environ. Sci. Technol. 33, 4514–4521.

Daughney, C.J., Fein, J.B., Yee, N., 1998. A comparison of the thermodynamics of metal adsorption onto two common bacteria. Chem. Geol. 144, 161–176.

Fein, J.B., 2000. Quantifying the effects of bacteria on adsorption reactions in water–rock systems. Chem. Geol. 169, 265–280.

Fein, J.B., Daughney, C.J., Yee, N., Davis, T.A., 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. Geochim. Cosmochim. Acta 61, 3319–3328.

Fein, J.B., Martin, A.M., Wightman, P.G., 2001. Metal adsorption onto bacterial surfaces: development of a predictive approach. Geochim. Cosmochim. Acta. 65, 4267–4273.

Fein, J.B., Boily, J.-F., Yee, N., 2004. Potentiometric titrations of *Bacillus subtilis* cells and a comparison of modeling approaches. Geochim. Cosmochim. Acta (submitted for publication).

Fowle, D.A., Fein, J.B., 2000. Experimental measurements of the reversibility of metal–bacteria adsorption reactions. Chem. Geol. 168, 27–36.

Haas, J.R., Dichristina, T.J., Wade Jr., R., 2001. Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. Chem. Geol. 180, 33–54.

He, L.M., Tebo, B.M., 1998. Surface charge properties of and Cu(II) adsorption by spores of the marine *Bacillus* sp. strain SG-1. Appl. Environ. Microbiol. 64, 1123–1129.

Ledin, M., Drantz-Rulcker, C., Allard, R., 1999. Microorganisms as metal sorbents: comparison with other soil constituents in multi-compartment systems. Soil Biol. Biochem. 31, 1639–1648.

Martinez, R.E., Smith, D.S., Kulczycki, E., Ferris, F.G., 2002. Determination of intrinsic bacterial surface acidity constants using a Donnan shell model and a continuous pK_a distribution method. J. Colloid Interface Sci. 253, 130–139.

Mullen, M.D., Wolf, D.C., Ferris, F.G., Beveridge, T.J., Flemming, C.A., Bailey, G.W., 1989. Bacterial sorption of heavy metals. Appl. Environ. Microbiol. 55, 3143–3149.

Plette, C.C., Van Riemsdijk, W.H., Benedetti, M.F., Van der Wal, A., 1995. pH dependent charging behavior of isolated cell walls of a gram-positive soil bacterium. J. Colloid Interface Sci. 173, 354–363.

Plette, C.C., Benedetti, W.H., Van Riemsdijk, M.F., 1996. Competitive binding of protons, calcium, cadmium, zinc to isolated cell walls of a gram-positive soil bacterium. Environ. Sci. Technol. 30, 1902–1910.

Tornabene, T.G., Edwards, H.W., 1972. Microbial uptake of lead. Science 176, 1334–1335.

Tortell, P.D., Maldonado, M.T., Granger, J., Price, N.M., 1999. Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiol. Ecol. 29, 1–11.

Warren, L.A., Ferris, F.G., 1998. Continuum between sorption and precipitation of Fe (III) on microbial surfaces. Environ. Sci. Technol. 32, 2331–2337.

Westall, J.C., 1982. FITEQL, a computer program for determination for chemical equilibrium constants from experimental data. Version 2.0. Report 82-02. Dept. Chem., Oregon St. Univ., Corvallis, OR, USA.

Wong, P.K., Lam, K.C., So, C.M., 1993. Removal and recovery of Cu(II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11. Appl. Microbiol. Biotechnol. 39, 127–131.

Xue, H.B., Stumm, W., Sigg, L., 1988. The binding of heavy metals to algal surfaces. Water Res. 22, 917–926.

Yee, N., Fein, J.B., 2001. Cd adsorption onto bacterial surfaces: a universal adsorption edge? Geochim. Cosmochim. Acta 65, 2037–2042.